REMARKS

Introductory Comments

Reconsideration of the above-identified application in view of the above amendments and foregoing arguments is respectfully requested.

Claims 1, 3-9 and 11-15 are pending and under consideration. The specification and claims 1, 5-9 and 11-15 have been amended. No new matter has been added as a result of these amendments. Claims 2 and 10 have been deleted.

Priority

The Examiner states that Applicant has not complied with conditions set forth under 35 U.S.C. § 119(e) by not providing a paragraph in the specification indicating that the application is a 371 of PCT/US00/12463 and referring to the Provisional U.S. Patent Application. Applicant has amended the specification to include this information.

Applicant submits that all of the informalities as stated by the Examiner have been addressed and respectfully requests the recognition of the priority claim.

Rejection of Claims 1-15 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-15 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Specifically, the Examiner asserts that 1) the claims are incomplete since the claims do not include the final result, the transformed *Allium* plant, tissue or callus, 2) the word "species" is improperly recited since it is not the species that is being transformed but the plant, tissue or callus instead, 3) the word "gene" is improperly recited since it is the not the gene but the DNA of interest that is used, 4) the word "material" is

redundant, 4) "EPSPS" is an acronym or abbreviation and should be spelled out, and 5) the word "about" is indefinite. The Examiner has suggested alternate recitations of claim language in order to overcome the rejection.

Applicant thanks the Examiner for her suggestions and has amended the claims to recite the language suggested by the Examiner. The phrases "plant or plant tissue", "embryogenic callus" and "DNA of interest" have been added to the claims instead of merely deleting the words "species" or "genes" so that these words retain antecedent support in the dependent claims. Also, Applicant notes that in certain portions of the specification, the abbreviation for "EPSPS" was incorrectly referred to as "5-enolpyruvyl-3-phosphate synthase". This typographical error has been corrected and the correct term "5-enolpyruvylshikimate-3-phosphate synthase" inserted instead. Support for this amendment can be found on page 11, lines 16-17.

Accordingly, Applicant respectfully requests withdrawal of the rejection of claims 1-15 under 35 U.S.C. § 112, second paragraph.

Rejection of Claims 8 and 15 Under 35 U.S.C. § 101

Claims 8 and 15 are rejected under 35 U.S.C. § 101 because the claimed invention is directed to non-statutory subject matter. Specifically, the Examiner states that a transformed species as claimed in claim 1 and 9 and the progeny thereof may contain wild-types.

Applicant has amended the claim to modify the word "species" as suggested by the Examiner in the claims, and to recite the phrase "under selective conditions" in claims 1, 8, 9 and 15 such that the claims do not read on the wild-type plants and the progeny thereof. Applicant thanks the Examiner for her suggestions.

Accordingly, Applicant respectfully requests withdrawal of the rejection of claims 8 and 15 under 35 U.S.C. § 101.

Rejection of Claims 1-15 Under 35 U.S.C. § 112, First Paragraph

Claims 1-15 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement because the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Specifically, the Examiner has provided the following arguments:

a) Regarding all *Allium* species as claimed, the Examiner states "Applicant has not provided a single working example of *Agrobacterium* transformation of *Allium cepa* using embryogenic callus derived from immature embryos or flower buds. Applicant's prophetic examples are detailed but lack showing of exemplified transgenic *Allium cepa* plant, material, plants or progeny. Applicant does not disclose which tissue sources, in combination with which specific treatment protocol and selection conditions function as desired in the claimed invention. Applicant has provided no guidance on how to predictably eliminate inoperable embodiments from a virtual *ad infinitum* of possibilities other than by random trial and error, which is excessive experimentation and an undue burden."

b) Regarding explants, the Examiner states "Applicant specifies no specific explant source for the embryogenic callus." The Examiner then argues that since plant transformation procedures employing plant tissue culture are unpredictable, early attempts have failed. The Examiner also states that Applicant has not disclosed the tissue sources and specific treatment protocols or the selective conditions, or how to eliminate inoperable embodiments; and

c) Regarding modified EPSPS genes, the Examiner states that "Since Applicant does not require that the modified gene have [a] function, myriads of different modifications, combinations and permutations of modifications can be made." Again, the Examiner argues that Applicant has provided no guidance in order to eliminate inoperable embodiments other than by random trial and error, requiring excessive experimentation and undue burden.

Applicant respectfully traverses the rejection based on these arguments. Applicant will address each of the above arguments herein.

The Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *Manual of Patent Examining Procedure* Section 2164.04, 8th Edition (Revision 2, May 2004). A specification that contains a teaching of the manner and process of making and using an invention in terms that correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. Section 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein that are relied on for enabling support. *Id.* As the CCPA stated in *In re Marzocchi,* 439 F.3d 220, 224, 169 USPQ 367, 370 (1971), "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." *Id.*

Regarding argument a), first, Applicant would like to remind the Examiner that compliance with the enablement requirement of 35 U.S.C. Section 112, first paragraph, does not turn on whether or not an example is disclosed in a specification. *See Manual of Patent Examining Procedure,* Section 2164.02, 8th Edition (Rev. 2, May 2004). Second, Applicant would like to point out that claims 1 and 9 have been amended to recite that the embryogenic callus material is from an *Allium cepa* or *Allium fistulosum*, therefore, not all *Allium* species are being claimed as asserted by the Examiner. Third, Applicant is confused as to the Examiner's statement that Applicant has not provided a single working example of *Agrobacterium* transformation of *Allium cepa* using embryogenic callus derived from immature embryos or flower buds and traverses the rejection based on this reasoning. As stated on page 6 of the Examiner's Office Action, Applicant's examples are detailed. Page 3, lines 1-6 clearly states that the

invention employs nodular embryogenic callus material and is derived from immature embryos or from flower buds. The immature flower buds can be obtained from unopened umbels from an onion. While pages 2-8 of the specification provide much information on the method as claimed, Examples 1-3 on pages 9-15 and Tables A and B provide even greater detail.

Page 9, lines 3-4 states that immature embryos from onion, specifically *Allium cepa* or *Allium fistulosum*, were isolated under a dissecting microscope from approximately 14 day post pollination flowers. Page 11, lines 4-8 specifically states "Callus material used in this experiment was initiated from immature embryos proprietary *Allium cepa* breeding material owned by Seminis Vegetable Seeds, Inc. Pollinated flowers were sent from Las Cruses, New Mexico to Woodland, California and immature embryos were isolated, using the procedures described in Example 1a from 11 proprietary *Allium cepa* lines (emphasis added)." On page 11, lines 15-18, it is stated "Experiment 212 used immature embryo derived callus of a proprietary *Allium cepa* line. Two selected callus lines which were transformed were regenerated from this experiment aided by the use of a regenerating embryogenic callus line as the initial tissue source." Applicant submits that these passages clearly disclose that the *Allium* species, such as *Allium cepa* and *Allium fistulosum* are disclosed and exemplified in the working examples provided in the specification. An example of the source of these species was disclosed to come from Seminis Vegetable Seeds, Inc., the assignee of the present invention.

The Examiner also questions the specific treatment protocol, selection conditions and how to eliminate inoperable embodiments, in her arguments with respect to a). These arguments are addressed in the below paragraphs and in Applicant's remarks in connection with argument c).

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *Manual of Patent Examining Procedure,* Section 2164.01, 8th Edition (Rev. 2, May 2004) (hereinafter "*MPEP*"). There are several factors to be considered when determining whether there is sufficient evidence to support a determination that a

specification does not satisfy the enablement requirement and whether any necessary experimentation is "undue". These factors have been described in *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) and include: (a) the quantity of experimentation necessary, (b) the amount of direction or guidance present; (c) the presence or absence of working examples, (d) the nature of the invention, (e) the state of the prior art, (f) the predictability or unpredictability of the art; (g) the breadth of the claims. In the Office Action, the Examiner went into detailed analysis regarding only one of the *Wands* factors, namely, the breadth of the claims. Applicant will now address each one of these factors.

First, with respect to factor (a), Applicant submits that the quantity of experimentation is not unreasonable or necessarily high for the claimed invention.

Claim 1, a method claim, comprises at least two (2) steps. One step involves contacting an embryogenic callus from a plant of an *Allium cepa* or *Allium fistulosum* with a bacterium belonging to the genus *Agrobacterium* which contains a DNA of interest from a heterologous gene. Another step involves obtaining a transformed *Allium cepa* or *Allium fistulosum* embryogenic callus under selective conditions.

Claim 9, also a method claim, comprises at least three (3) steps. One step involves culturing immature embryos or flower buds from a plant of an *Allium cepa* or *Allium fistulosum* species on an initiation medium for a period of from 2 months to 6 months until an embryogenic callus forms the embryos or flower buds. Another step involves transferring the embryogenic callus to a coculture medium and contacting the embryogenic callus with a suspension of *Agrobacterium rhizogenes* or *Agrobacterium tumefaciens* containing a DNA of interest from a heterologous gene. Another step involves obtaining a transformed *Allium cepa* or *Allium fistulosum* embryogenic callus under selective conditions.

Applicant submits that assuming *arguendo*, that any experimentation is required to practice the claimed invention, that the quantity of experimentation is

not necessarily high as a reasonable amount of guidance for the claimed invention has been provided in the specification and working examples. Specifically, the working examples describe in detail how embryogenic callus material can be obtained from immature embryos or flower buds and then how this embryogenic callus can then be contacted with *Agrobacterium* containing a DNA of interest. Although no regenerated transformed plants are described in the working examples, Applicant submits that the techniques for obtaining regenerated transformed plants as a result of *Agrobacterium* transformation is routine and well known to those skilled in the art. In fact, the specification on page 8, lines 24-26, describes that "[T]ransformed plants containing the heterologous gene described herein can be identified using techniques known in the art such as Northern or Southern Blotting or polymerase chain reaction."

With respect to factor (b), Applicant has provided adequate direction and guidance of the claimed invention in the specification. Applicant has provided working examples describing the *Agrobacterium* transformation of *Allium cepa* and plants resulting from a cross of *Allium fistulosum x Allisum cepa* using embryogenic callus derived from immature embryos or flower buds. Applicant has also described that transformed plants containing the heterologous gene can be identified using techniques that are well-known to those skilled in the art, such as Northern or Southern blotting or polymerase chain reaction). Thereupon, Applicant submits the one of ordinary skill in the art would understand and appreciate the direction and guidance provided in the specification with respect to the claimed invention.

With respect to factor (c), as discussed previously, there is a presence of working examples provided in the specification instead of an absence of working examples.

With respect to factor (d), the nature of the invention is not overly complex and is not considered a new or emerging technology. In *Enzo Biochem., Inc. v. Calgene, Inc.,* 188 F. 3d 1362 (Fed. Cir. 1999), the Federal Circuit stated that consideration of an invention in a new technology impacts on how the *Wands* factors are evaluated. The Court also states that the unpredictable arts include

areas of technology and research that require further development and understanding. *Id.* at 1372. Applicant submits that although other areas of biotechnology are new and unpredictable, the specific area of technology which Applicant's invention pertains is not overly complex such that undue experimentation is likely required to carry out Applicant's invention. Although the application of the claimed steps are novel and non-obvious as a whole, the individual steps, such as for example, contacting an embryogenic callus from a plant of an *Allium cepa* or *Allium fistulosum* with a bacterium belonging to the genus *Agrobacterium* which contains a DNA of interest from a heterologous gene and obtaining a transformed *Allium cepa* or *Allium fistulosum* embryogenic callus under selective conditions, as recited in claim 1, would be recognizable and understood by one of ordinary skill in the art.

With respect to factor (e), there has been a description of successful transformation of onion by Eady (Eady, C.C., Weld R.J. & Lister, C.E. Transformation on onion, *Allium cepa L., Proc. Nat. Onion Research Conference,* Sacramento, CA USA, Dec. 10-12, 1998), using *Agrobacterium* with a kanamycin selectable marker and a green florescent protein scoreable marker. Nonetheless, Applicant's invention is novel and non-obvious. Nonetheless, in view of the state of the prior art and the amount of information provided in the specification, one of ordinary skill in the art would be able to make and/or use the invention.

With respect to factor (f), the predictability or unpredictability of the art, the *MPEP* Section 2164.03 states that "the predictability or lack thereof" in the art "refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. As discussed above with respect to factor (e), there has been a successful transformation of onion by Eady (Eady, C.C., Weld R.J. & Lister, C.E. Transformation on onion, *Allium cepa L., Proc. Nat. Onion Research Conference,* Sacramento, CA USA, Dec. 10-12, 1998), using *Agrobacterium* with a kanamycin selectable marker and a green florescent protein scoreable marker. Thereupon, there is predictability in transforming onion using *Agrobacterium*, although Applicant's invention is novel and non-

obvious. As noted above, Applicant has provided direction and guidance for each of the steps of its claimed methods. Although no method has been performed using all of the steps as claimed, there is no reason for one of ordinary skill in the art to question the methods as disclosed in the specification. Applicant further submits that the working examples in the specification provide adequate detailed information that even assuming there were any unpredictability in the art, that the amount of unpredictability is acceptable.

With respect to factor (g), the breadth of the claims is not overly broad. As noted above, Applicant has amended the claims to recite that the embryogenic callus material is from an *Allium cepa* or *Allium fistulosum*.

Regarding argument b), Applicant traverses the rejection based on this reasoning. As quoted above, the specification clearly discloses the plant tissue is derived from immature embryos. Starting on page 3, line 8, the explants and procedures and selection conditions are disclosed.

"Once the immature embryos or flower buds are obtained, they are placed on a callus initiation medium such as the initiation medium described in Table A as media number one (#1) and kept under appropriate environmental conditions, specifically, in the dark and at a temperature between about 25°C to about 30°C, to allow the formation of callus. Other initiation media which induce the formation of callus which are well known in the art, can also be used. For example, any salt formulation media, such as but not limited to, Murshige and Skoog (MS) ... Growth 7:53 (1943), which contain a high concentration of auxins (such as indole acetic acid (IAA)), 2,4-dichlorophenoxy acetic acid, picloram, indole butyric acid (IBA) as well as carbon source (such as glucose, sucrose, etc) can be used." (Page 3, lines 8-23.)

"After about two (2) to six (6) months, nodular embryogenic callus forms on the embryos or flowers. The callus is maintained by subculturing every four (4) weeks, keeping the culture in the dark at a temperature between 25°C to about 30°. During this period, any tissue which is not nodular embryogenic callus is removed from the culture. Specifically, the removal of brown or smooth textured tissue and of tissue with anthocyanin or sticky exudates facilitates the

development of the nodular embryogenic callus. The nodular embryogenic callus is the material suitable for transformation with Agrobacterium." (Page 3, line 25 to page 4, line 2.)

"For regeneration, the nodular embryogenic callus is transferred to a regeneration medium such as the regeneration medium provided for in Table A as media number two (#2) and is placed under Cool White fluorescent light for about fourteen (14) to about eighteen (18) hours per day at a temperature between about 25°C to about 30°C. Other regeneration media which are well known in the art can also be used. For example, any salt formulation medium, such as, but is not limited to, Murshige and Skoog (MS), B-5, Heller, White, which contains low levels of cytokinins (such as benzylaminopurine (BA), kinetin, 6-dimethyallyaminopurine (2IP) and carbon source (such as glucose, sucrose, etc.)) can also be used." (Page 4, lines 4-12.)

The procedure of how the heterologous gene that is used to be expressed in onion and used to construct an expression cassette which is introduced into onion is stated starting on page 6, line 8. The elements of the expression cassette are described on page 6, line 10 to page 7, line 22.

Starting on page 7, line 24, the selective condition used in the transformation process and the medium used in this process are described. "The nodular embryogenic callus material prepared as described above is then contacted with the *Ti* or *Ri* plasmid of *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* which contains the expression cassette with the heterologous gene. After the embryogenic callus material is contacted with the *Agrobacterium*, it is then incubated for about two (2) to about four (4) days at a temperature of about 20°C to about 25°C in the dark. After the incubation period, the *Agrobacterium* is removed or disinfected such as by scraping callus tissue into a dish with wash media, such as the wash medium described in Table B, agitating it and then removing the wash medium." (Page 7, lines 24-31.) "After removal of the Agrobacterium, the washed embryogenic callus material is transferred to a selection medium, such as the selection medium described in Table A as media number four (#4). Other selection media, which are well

known in the art, such as media containing the antibiotic kanamycin, can also be used. The callus cultures are grown under Cool White fluorescent light for about 14 to about 18 hours per day at a temperature about 25°C to about 30°C." (Page 8, lines 1-7.) "After about thirty (30) days, the callus is subcultured onto a second higher selection media, such as the selection medium described in Table A as media number five (#5), for all following transfers. Selection transfers are done every four (4) weeks per subcultures." (Page 8, lines 9-12.) "Any remaining callus which is living and is producing embryos or plants is then transferred to the rooting media in 0.05 mM glyphosate which is described in Table A as media #6 for final regeneration. Other media which are well known in the art can also be used. The regeneration shoots are grown under Cool White fluorescent light for about 14 to about 18 hours per day at a temperature about 25°C to about 30°C. Regeneration and rooted shoots are then transplanted into pots filled with soil under high light intensity, such as 1000 foot candles, and at near 100% relative humidity, such as by covering the pots with plastic." (Page 8, lines 14-21.)

Applicant submits that these passages clearly disclose what the explants, media and selective conditions used in the culturing and transformation process are. Every step of every culture described in the specification states what the media, the time and temperature are. Specific media are listed in Table A and B as indicated above. Other specific details are described in the working Examples 1-3. Thus, as shown above, Applicant has disclosed the tissue sources and specific treatment protocols or selective conditions. As to how inoperable embodiments are eliminated, the Examiner is directed to page 8, lines 9-12 where it is disclosed that selection transfers are done every four weeks per subculture, page 3, lines 25-30 where it is disclosed that any tissue which is not nodular embryogenic callus is removed and exactly what kind of tissue via examination is removed, and page 14, line 27 to page 15, line 2 where it is disclosed the selection procedures.

Regarding the Examiner's argument c), Applicant traverses the rejection based on this argument. Starting on page 4, line 14, the specification clearly

describes the target gene and the function of the gene. "The heterologous gene used in the method of the present invention encodes the expression of a protein, such as 5-enolpyruvylshikimate-3-phosphate synthase enzyme [(EPSPS)], which conveys resistance to the glyphosate herbicide. The desired heterologous gene to be inserted into onion can be isolated using molecular biology techniques which are well known in the art or can be produced synthetically using molecular biology techniques which are also well known in the art." (Page 4, lines 14-20.)

"As is well known in the art, glyphosate inhibits the shikimic acid pathway which leads to the biosynthesis of aromatic compounds including amino acids, plant hormones and vitamins. Specifically, glyphosate curbs the conversion of phosphoenolpyruvic acid and 3-phosphoshikimic acid to 5-enolpyruvyl-3 phosphoshikimic acid by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (hereinafter referred to as 'EPSPS' or 'EPSP synthase'). It is well known that glyphosate-tolerant plants can be produced by inserting into the genome of the plant the capacity to produce a higher level of EPSP synthase in the chloroplast of the cell which enzyme is preferably glyphosate-tolerant." (Page 4, line 22 to page 5, line 2.)

Starting on page 5, line 4, how the gene is modified is disclosed. These genes are modified to transform plants to make plants which are tolerant to glyphosate herbicides. For example, the genes can be modified using methods such as mutating the aroA gene to affect EPSPS, mutating the gene to allow chloroplast transit peptides to be transported into the chloroplast of the cell of the plant, or substituting alanine for glycine or other genetic modifications for different benefits. (Page 5, line 4 to page 6, line 6.)

Other ways to modify EPSPS and their known functions are described on page 7, lines 5-22, page 11, lines 4 to page 12, line 12 and page 13, line 4 to page 14, line 2. Applicant submits that the specification clearly discloses the different ways the gene is modified and their known functions or benefits such that no random trial and error requiring excessive experimentation and undue burden is needed.

Also, as discussed on page 11, line 18, U.S. Patent No. 5,633,435 discloses a modified EPSPS gene.

Additionally, Applicant would like to point out that claims 6 and 13 have been amended to recite that the modified EPSPS gene, when expressed, encodes an enzyme that confers resistance to the herbicide glyphosate.

The Examiner cites a source stating that plant transformation procedures employing tissue culture are unpredictable and early attempts have failed for her basis in rejecting the claims. Applicant respectfully submits that this basis for rejecting the claims is improper.

Therefore, Applicant respectfully requests withdrawal of this rejection of claims 1-15 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 8 and 15 Under 35 U.S.C. § 102(b)

Claims 8 and 15 are rejected under 35 U.S.C. § 102(b) as being anticipated by Dommisse *et al.*, "Onion is a Monocotyledonous Host for *Agrobacterium*", *Plant Science*, Vol. 69 (1990), pages 249-257 (herein "Dommisse").

The Examiner states that Dommisse anticipates the claimed invention because Dommisse teaches *Agrobacterium* mediated transformation of *Allium cepa* bulbs, which *Agrobacterium* contains opine synthase genes heterologous to *Allium* (pages 254-44). Applicant respectfully traverses the rejection.

For a claim to be rejected under 35 U.S.C. Section 102(b), each and every element of the claimed invention must be disclosed in a prior art reference. Applicant respectfully reminds the Examiner that claims 8 and 15 are product claims (i.e. claims to *Allium* plants or plant tissues) that are produced by the methods of 1 and 9 respectively.

Dommisse discloses bulbs and leaves of onions inoculated with *Agrobacterium* (page 249 and abstract) and subsequently tumor growth was found immediately surrounding the inoculation sites (page 250, the paragraph bridging the left and right columns). Dommisse does not disclose or suggest using embryogenic callus material from an *Allium* species and contacting the

same with an *Agrobacterium* organism containing a DNA of interest from a heterologous gene. Instead, the present invention provides a method for an effective transformation of an *Allium cepa or Allium fistulosum* plant in contrast to Dommisse, who teaches the local infection of an *Allium* plant with *Agrobacterium.* Dommisse simply does not disclose the use of embryogenic callus material for such a purpose.

Therefore, because Dommisse fails to teach each and every element of the claimed invention, Applicant respectfully requests withdrawal of the rejection of claims 8 and 15 under 35 U.S.C. § 102(b) as being anticipated by Dommisse *et al.*

CONCLUSION

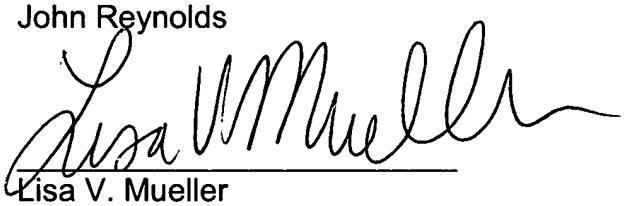
Applicant respectfully submits that the claims comply with the requirements of 35 U.S.C. Sections 101, 112 and 102. Accordingly, a Notice of Allowance is believed in order and is respectfully requested.

Should the Examiner have any questions concerning the above, she is respectfully requested to contact the undersigned at the telephone number listed below. If the Examiner notes any further matters which the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

If any additional fees are incurred as a result of the filing of this paper, authorization is given to charge deposit account no. 23-0785.

Respectfully submitted,

John Reynolds



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